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GROWTH POTENTIALITIES OF VEGETATIVE BUDS ON APPLE TREES[1]

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INTRODUCTION

Evidence of a functional relationship between leaves on apple shoots and their axillary buds has been established by defoliation and defloration experiments. Since leaf area is considered an essential factor in controlling blossom bud formation,[2 3] a similar relationship should be expected to exist between leaf area and the potency of vegetative buds. Size of leaves and buds also has been considered as dependable measures of the vigor of the tree.[4] The actual size relations between leaves and their axillary buds, however, have not yet been determined under natural developmental conditions of the shoot. The following study was made to determine (1) the relations between leaf area, bud size, and leaf drop on the current year's shoot, (2) the relationship of lateral bud sizes on 1-year-old twigs to their subsequent vegetative growth, and (3) the sequence of bud breaking.

MATERIALS AND METHODS

For this study two varieties of apple trees were chosen which under the conditions of the college orchard showed rather marked differences in fruiting characters, the Stark, whose buds break freely and which comes into bearing fairly early, and the Northern Spy which showed the opposite features, i. e., poor breaking and late-bearing qualities. Occasionally, also, other varieties were examined. The trees were about 20 years old. Part of the study is based on close observation of representative shoots of both Stark and Northern Spy. These were selected from all sides of the lower third of the tree. Some had made a late seasonal or secondary growth.

Shoot length was measured with a ruler to the closest 0.5 cm. and diameter with a micrometer caliper graduated to 0.1 mm. Bud size was calculated by multiplying length and width which were measured

[1] Received for publication July 31, 1947. Journal Article No. 968 (n. s.) from the Michigan Agricultural Experiment Station.

[2] ROBERTS, R. H. APPLE PHYSIOLOGY. GROWTH, COMPOSITION, AND FRUITING RESPONSES IN APPLE TREES. Wis. Agr. Expt. Sta. Res. Bul. 68, 72 pp., illus. 1926.

[3] STRUCKMEYER, B. E., and ROBERTS, R. H. INVESTIGATIONS ON THE TIME OF BLOSSOM INDUCTION IN WEALTHY APPLE TREES. Amer. Soc. Hort. Sci. Proc. 40: 113–119, illus. 1942.

[4] BLAKE, M. A., EDGERTON, L. J., and DAVIDSON, O. W. STANDARDS FOR JUDGING THE GROWTH STATUS OF APPLES IN NEW JERSEY. N. J. Agr. Expt. Sta. Bul. 715, 36 pp., illus. 1945.

with a steel spacing divider. Leaf areas (A) were determined either by directly tracing blueprints with a polar planimeter or by measurements of greatest length (L) and width (W), and multiplying the product of these two measurements by the proper leaf factor coefficient (C) according to the formula $A=C\ (WL)$. The leaf factor for each variety was obtained by comparing the results of measurements of about 100 leaves of various sizes by both contour tracing and length and width measurements, and calculating it according to the formula $C=\frac{\Sigma A(WL)}{\Sigma(WL)^2}$. The leaf factor for Stark was 0.7031 and for Northern Spy 0.8194, the standard errors being 0.1084 and 0.0640 respectively ($\sigma=\frac{\Sigma A^2-C.\Sigma A(WL)}{n}$. Series of buds destined for microscopical examination were fixed in formalin-propionic acid, dehydrated by the tertiary butyl alcohol method, and embedded in Bioloid paraffin. Safranine and light green or Delafield haematoxylin were the stains used.

Only summarized data are presented in the tables.

THE GENERAL DEVELOPMENT OF LEAF AND BUD SIZES ON THE SHOOT

The first two or three leaves that develop on the shoot present typical transitional forms.[5] They are followed by another group of two or three leaves which are normally shaped. None of these are likely to have visible buds in their axils, but later-formed leaves usually have plainly visible axillary buds. Though successive young leaves appearing at the tip of the shoot go through essentially the same size changes, the size of their axillary buds when they first become visible at the base of the petiole varies greatly during different developmental stages of the shoot.

Contradictory to the generally held opinion of a direct relationship existing between leaf and bud sizes, a constantly inverse relationship between these sizes was observed on Stark, Northern Spy, and numerous other varieties during the first month of growth, in both the 1945 and 1946 seasons. The phenomenon was typical after eight or nine leaves of the new shoot were formed. The partially unfolded leaf near the apex of the shoot subtended a much larger bud than that in the axil of an already expanded lower leaf which had a distinctly larger area. The picture of a Stark shoot taken May 7 illustrates this condition (fig. 1). When the leaves were removed on that date, the five basal nodes had no visible buds; from the sixth to the ninth node bud sizes gradually increased, though leaf sizes were progressively smaller; the younger buds were larger than the older ones. The ninth leaf, though comparatively small, subtended the largest bud.

A series of successive measurements made during the first 2 months of growth which illustrate the relations between bud size and leaf area, are presented in table 1. On April 19, when the shoot length of Stark was 5 cm. the maximum leaf area was at the sixth node, the maximum bud size at the ninth node. As growth continued the maxima tended

[5] MacDaniels, L. H., and Cowart, F. F. THE DEVELOPMENT AND STRUCTURE OF THE APPLE LEAF. N. Y. (Cornell) Agr. Expt. Sta. Mem. 258, 29 pp., illus. 1944.

to approach each other, until they coincided at about the middle of June; thereafter, a more direct correlation of leaf and bud sizes prevailed, the older buds being larger and having larger subtending leaves than the newly formed buds near the tip of the shoot.

A comparable condition was found in Northern Spy, only delayed by about 1 or 2 weeks because of the later "breaking" of this variety. The spatial differences of leaf and bud size maxima on Northern Spy

FIGURE 1.—Shoot of Stark apple on May 7, 1945, shows largest buds in axils of youngest leaves near the apex. Leaves are numbered according to their natural acropetal sequence.

were even greater than on Stark during the first 3 weeks of the shoot's development. On April 27, the largest leaf was located at the fifth node, while the maximum bud size was at the tenth node.

These inequalities in the developmental cycles of leaves and axillary buds on shoots seem to have passed unnoticed in the literature. They may be interpreted as an indication of different factors controlling leaf and bud growth during their early stage of development.

After the developmental phase of the shoot is reached where the largest buds are found in the axils of the largest leaves, the size of both

TABLE 1.—*Size relation between leaf area and axillary bud in the spring*[1]

STARK

Date	Shoot length	Shoot diameter	Leaf area and bud size	Node No.—																		
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Cm.	*Cm.*																				
Apr. 19	5.0	0.50	Leaf area.....cm.²	2.57	8.39	10.32	11.61	18.39	[1] 20.65	12.35	9.34	5.8										
			Bud size.....mm.²							0.5	1.0	[1] 1.50										
May 7	6.5	.53	Leaf area.....cm.²	5.81	11.61	12.90	18.71	19.35	24.52	[1] 25.16	9.03	4.52	1.29									
			Bud size.....mm.²						.5	1.5	2.25	[1] 3.0	1.0									
May 21	8.5	.64	Leaf area.....cm.²	3.23	9.03	16.78	20.0	16.78	14.19	18.07	[1] 28.39	5.16	9.03	5.16	1.29							
			Bud size.....mm.²				.5	1.8	4.0	5.72	5.76	5.0	[1] 6.3	2.7	2.8							
June 19	26.0	.83	Leaf area.....cm.²	5.16	13.55	15.48	21.29	20.0	16.78	13.55	25.16	21.98	28.39	[1] 39.36	40.0	34.84	34.19	[1] 42.23	34.84	16.13	10.22	2.58
			Bud xize.....mm.²					1.20	1.80	5.40	6.76	8.40	9.0	[1] 13.65	12.25	10.50	13.65	[1] 13.65	6.72	7.80	5.0	1.0

NORTHERN SPY

Apr. 27	3.6	.42	Leaf area.....cm.²	1.95	5.31	8.11	8.13	[1] 9.10	6.37	4.60	3.41	2.52	1.40									
			Bud size.....mm.²									.10	[1] .80									
May 17	5.5	.45	Leaf area.....cm.²	.65	4.47	5.16	5.81	12.90	[1] 18.71	14.88	14.19	11.61	5.48	2.57								
			Bud size.....mm.²							.10	1.0	1.00	[1] 1.50	.50								
May 28	8.0	.50	Leaf area.....cm.²	1.94	3.87	7.10	10.07	11.61	9.03	14.19	[1] 15.48	7.30	5.81	3.87	1.29							
			Bud size.....mm.²							.10	1.0	1.44	[1] 2.35	1.8	.12							
June 12	13.0	.56	Leaf area.....cm.²	1.65	2.58	5.80	6.45	7.74	9.67	15.48	20.0	20.0	[1] 25.16	9.67	7.09	7.74	5.16	2.58				
			Bud size.....mm.²										1.56	4.18	[1] 6.25	3.99	2.35	1.21				
June 20	18.0	.60	Leaf area.....cm.²	3.23	3.87	12.26	12.90	16.13	20.65	20.0	26.45	25.81	26.78	25.49	29.03	[1] 36.13	19.03	7.74				
			Bud size.....mm.²							1.0	2.25	3.6	6.09	7.29	5.72	[1] 7.84	5.4	.10				

[1] Maxima.

the buds and the leaves may vary greatly. There is, however, a tendency toward a gradual decrease in size of both until the shoot ceases to elongate. Only when the terminal growing point is prematurely halted in its further production of leaves and axis elongation, incident to drought or other interference, the apical leaves and buds may become the largest of the shoot. Simultaneously, considerable thickening of the axis takes place, resulting in less tapering from base to apex of the shoot. If growth proceeds slowly and continues for a long period, the shoot will, of course, be relatively long at the end of the growing season, and its median and submedian section will have larger leaves than will the apical portion. These growth characteristics, however, display a wide range of variation even on the same tree and during the same season.

A variation in the development of many shoots is occasioned by a resumption of active growth after a summer pause. This is often called second growth. It is characterized by a repetition of the normal sequence of development, but in a more or less abbreviated form. In 1945 a short drought in July was followed by heavy rains in August that enabled the terminal growing point to resume growth after it had ceased. In 1946 the almost complete lack of rain during July, August, and September terminated the normal shoot growth early in July. Late growth was rarely formed, and only on those shoots on which a few of the most apical leaves had been attacked by leafhoppers. Figure 2 portrays such a shoot of Fameuse on October 10. Harley [6] observed induction of a second and even third apical growth on spurs after partial defoliation. In both instances, apparently, a growth substance is activated subsequent to the injuries. The differences in length and diameter of the terminal shoots which were formed in two successive seasons are illustrated by the following data: In 1945 the mean length of the 25 Stark shoots was 36 cm. and the mean diameter 0.77 cm. The terminal buds of these shoots produced in 1946 a mean extension of only 20 cm. in length and 0.64 cm. in diameter. The 25 Northern Spy shoots reached in 1945 a mean length of 24 cm. with a mean diameter of 0.60 cm. and in 1946 a mean length of 16.7 cm. with a mean diameter of 0.56 cm. Consequently, the total bud number on the 1946 shoots was only slightly more than half that of the preceding year, the proportion being 510: 275 on Stark, 311: 177 on Northern Spy. However, the various bud size categories on the shoots were in both seasons distributed in similar proportions.

RELATION OF BUD SIZE TO NUMBER OF LEAF PRIMORDIA

If size indicates the degree of preparedness of the bud for its future growth, the question at once arises as to whether there is a corresponding degree of differentiation and development of its embryo structures. An examination of the number of leaf primordia present in the lateral shoot buds seemed the simplest practical way to determine the relation existing between external size and internal structure of buds. Counts of leaf primordia were made of each bud of six shoots that were

[6] HARLEY C. P., MAGNESS, J. R., MASURE, M. P., FLETCHER, L. A., and DEGMAN, E. S. INVESTIGATIONS ON THE CAUSE AND CONTROL OF BIENNIAL BEARING OF APPLE TREES. U. S. Dept. Agr. Tech. Bul. 792, 58 pp., illus. 1942.

FIGURE 2.—Second apical growth of Fameuse apple following leafhopper injury.

collected on July 23 and October 25 from Stark and on August 2 and October 25 from Northern Spy. The numerical determinations were made after leaf and bud sizes were recorded according to their natural acropetal sequence. The counts were made on median longitudinal sections. The figures which resulted from the measurements were plotted on semilogarithmic paper to symbolize the interrelations that

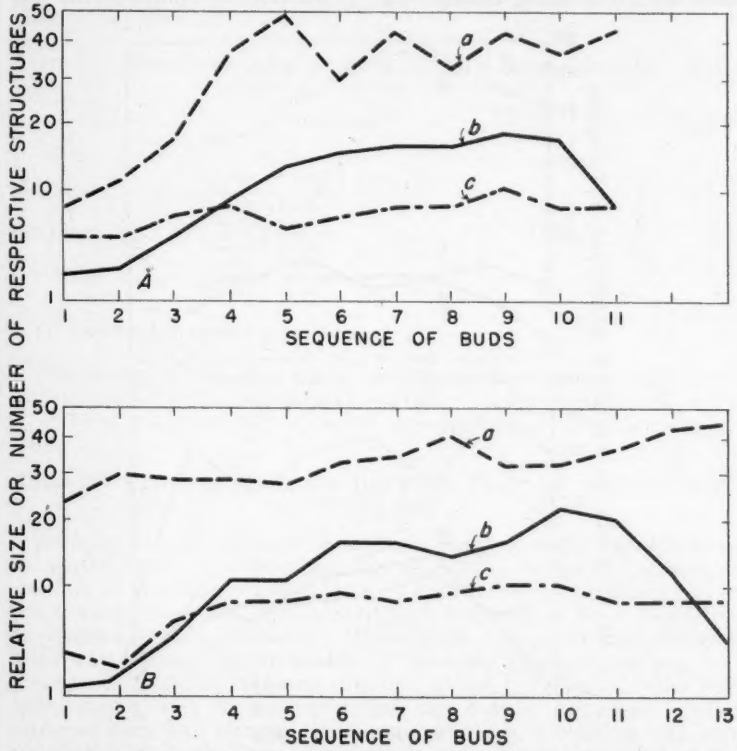

FIGURE 3.—Curves showing relations between area of subtending leaves in square centimeters (*a*), axilliary bud size in millimeters (*b*), and number of leaf primordia (*c*) on shoots of Stark apple. *A*, Shoot length 17 cm., diameter 0.48 cm.; July 23, 1946. *B*, shoot length 22 cm., diameter 0.87 cm.; October 25, 1946.

existed between the three series of magnitudes of each shoot. A few typical examples of these diagrams are shown in figures 3 and 4. The array of nodes from base to tip of the shoots is plotted as invariable on the arithmetic scale of the abscissa.

A total of 113 lateral buds and their subtending leaves were measured. In general, there is a fairly close correspondence between size of bud and number of leaf primordia, while the size of the associated subtending leaf often varies independently. In none of the cases were the maxima of all three magnitudes coordinated.

The maximum number of leaf primordia in lateral buds on July 23 ranged from 8 to 10 on Stark, from 7 to 8 on Northern Spy. On October 25, 10 was the maximum for both varieties, though the averages always were a little higher for Stark (6.7 and 6.34) than for Northern Spy (6.05 and 5.57). The number of leaf primordia in the terminal bud was only slightly higher than in the lateral buds. When the maximum among laterals was 8, the terminal bud had 10 leaf

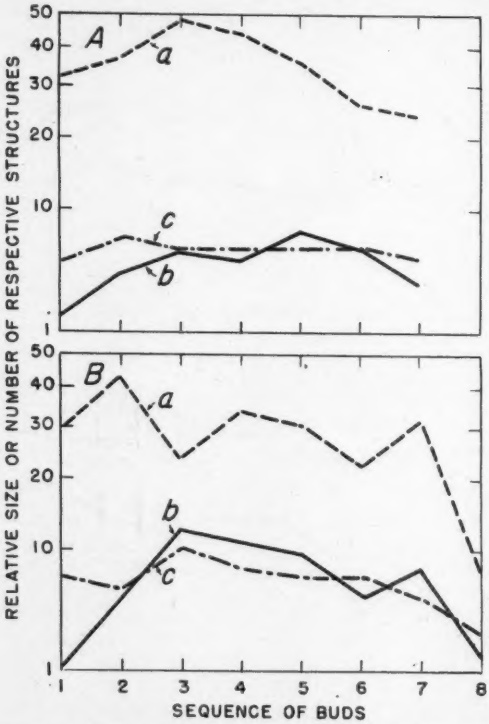

FIGURE 4.—Curves showing relations between area of subtending leaves in square centimeters (*a*), axillary bud size in millimeters (*b*), and number of leaf primordia (*c*) on shoots of Northern Spy apple. *A*, shoot length 19 cm., diameter 0.55 cm.; August 2, 1946. *B*, Shoot length 21 cm., diameter 0.63 cm.; October 25, 1946.

primordia; when it was 10, the terminal bud had 12. This numerical relation of leaf primordia between lateral and terminal buds was the same on both Stark and Northern Spy despite the smaller bud sizes of the latter variety. The average size of 6 terminal buds on Stark, on October 25, was 53.33 mm.^{2} ± .42; on Northern Spy it was 23.33 mm.^{2} ± 2.11.

Additional measurements of lateral buds and counts of their leaf primordia were made with material collected at random from Stark shoots on April 7 and June 9, 1944, and on March 15, 1945. Each

sample included 10 buds. Their sizes were graded as "large" when the bud length exceeded 4 mm., as "medium" when between 2 and 4 mm., and as "small" when bud length was less than 2 mm. The buds of April 7 and March 15 were mature laterals from 1-year-old twigs, those of June 9, immature buds from the current year shoots. They were embedded in paraffin and cross-sectioned with a microtome. The result of the count of leaf primordia for each group of these samples is presented in table 2.

TABLE 2.—*Mean number of leaf primordia in relation to bud size on Stark apple* [1]

Date	Size of buds	Mean number of primordia	Standard deviation	Standard error
Apr. 7, 1944	Large (>4 mm.)	7	0.82	±0.26
	Medium (2-4 mm.)	5.5	1.66	±.53
	Small (<2 mm.)	4	.82	±.26
June 9, 1944	Large (>4 mm.)	5.6	1.63	±.51
	Medium (2-4 mm.)	5.4	1.77	±.56
	Small (<2 mm.)	4.8	.95	±.30
Mar. 15, 1945	Large (>4 mm.)	8	.82	±.26
	Medium (2-4 mm.)	6	.75	±.27
	Small (<2 mm.)	4.6	1.63	±.51

[1] The total number of buds was 10 in each size group.

The figures within each set of bud groups show a reasonably good correlation between bud size and number of leaf primordia, the larger buds always having differentiated more primordia than the smaller buds.

GROWTH PERFORMANCE OF LATERAL BUDS IN RELATION TO THEIR SIZE

Each bud on the selected 25 terminal twigs of Stark was measured on April 2 and each of Northern Spy on April 9, before any noticeable swelling of the tissues had started. Observation began shortly after the terminal buds had opened and gave evidence of their vegetative or blossom-forming character. The growth activity of the individual buds was followed up at weekly or biweekly intervals, taking into account the date of breaking, the increase in spur leaf number and spur lengths, and the amount of leaf loss during the season. After terminal buds had formed, measurements of the remaining leaf area were taken, on August 26. There was practically no time at which the complete leaf area of the shoot could be obtained, since losses of leaves had already occurred before the leaves were fully developed in number and size. However, the belated measurement seems to give a fairly reliable picture of the original leaf production, since the amount of leaf drop within the single groups differs only by a small percent.

The data obtained from growth records of each lateral bud are analyzed in table 3. For convenience of analysis the original bud sizes of all shoots of the single varieties were assembled in four groups (A–D).

STARK

The four arbitrary sizes ranged for group A from 1 to 10 mm.,[2] for group B from 10 to 20 mm.,[2] for group C from 20 to 30 mm.,[2] and for group D from 30 to 40 mm.[2] The distribution of the 510

TABLE 3.—*Growth performance of lateral buds in relation to their size, on 25 terminal shoots, 1945* [1]

Buds and spur leaves	Stark (bud group)					Northern Spy (bud group)				
	A (1–10 mm.²)	B (10–20 mm.²)	C (20–30 mm.²)	D (30–40 mm²).	Total	A (0–1 mm.²)	B (1–5 mm.²)	C (5–10 mm.²)	D (10–15 mm.²)	Total
Total number of buds	[1] 191	170	129	20	510	42	110	[1] 136	23	311
Buds:										
Number active	39	[1] 116	113	18	286	1	16	[1] 70	14	101
Percent active	20	68	88	[1] 90		2.4	14.5	51.5	[1] 61	
Percent dormant	[1] 80	32	12	10		[1] 97.6	85.5	48.5	39	
Spur leaves:										
Number	179	609	[1] 717	131	1,636	5	65	[1] 347	74	491
Average per bud	4.6	5.3	6.3	7.3		*5	4.1	5.0	5.3	
Spur leaf area (cm.²) August 26:										
Total	1,092.89	3,906.84	[1] 5,062.26	767.59	10,829.58	23 12	458.97	[1] 3,057.71	812.31	4,352.11
Average per bud	28	34	45	43		*23	29	44	55	
Percent of leaf loss, August 26	[1] 56	53	51	47		*2	[1] 46	43	36	

[1] Maxima.
*Refer to single cases and have no significance.

buds among the several groups were: 191 or 36 percent in group A (small), 170 or 33 percent in group B, 129 or 25 percent in group C, and 20 or 4 percent in group D (large). A total of 224 buds (44 percent) remained dormant during the season, including those buds which after starting to break ceased growing without unfolding a leaf. Larger percentages of the small buds than of the larger ones remained dormant.

The total number of spur leaves produced by the 286 active buds amounted to 1,636. The average leaf number of the lateral shoots increased progressively from 4.6 to 7.3 with increasing bud size, indicating a direct relationship between parent bud size and leaf number of lateral growth.

By August 26, 48 percent of the leaves had dropped. Although the numerical differences of abscised leaves within the various bud categories are small, the amount of spur leaf drop distinctly shows an inverse relationship to the sizes of the parent buds. Groups A–D lost 56, 53, 51, and 47 percent, respectively. The area of the remaining leaves was 10,830 cm.² The averages of spur leaf area that is retained, however, follow leaf number, and increase progressively with the size of the parent buds, ascending from 28 cm.² to 43 cm.² with respect to bud sizes from A to D.[7]

NORTHERN SPY

The relations between parent bud size and growth capacity on the twigs of Northern Spy were found to be very similar to those on Stark. Because of their characteristically smaller size the bud classification for the four size groups was based on the following order of magnitude: 0 to 1 mm.² for group A, 1 to 5 mm² for group B, 5 to 10 mm.² for group C, and 10 to 15 mm.² for group D. The 25 twigs carried alto-

[7] The somewhat greater leaf area of the "C" size group (45 cm.²) apparently is an irregularity which can be disregarded, since only a few extraordinarily large leaves may have caused this result without altering the true general picture.

gether 311 lateral buds, of which 44 percent and 35 percent were of medium size, belonging to groups C and B respectively. Two hundred and ten buds (68 percent) of the total number remained dormant. Among the inactive buds 98 percent belonged to the smallest size (group A). The frequency of dormancy decreased with the increase in bud size. Among the remaining 101 growth-producing buds, those of group C contributed most of the spurs (69 percent), and subsequently led with leaf number and area. The mean of leaf number and leaf area per spur was directly related to the parent bud size. Although the average number of spur leaves differed only slightly, the average leaf area showed an increase from 23 cm.^{2} to 55 cm.^{2}, gradually ascending between the groups A and D. The leaf drop during the season decreased from groups A to D, being inversely correlated to bud size. Sixty-four percent of leaves in group D had persisted on August 26, and 54 percent of leaves in group B.

The data in table 3 indicate conclusively that (1) the smallest buds are predominant in number, but most of them remain inactive; (2) spur and spur leaf production is contributed mostly by medium-sized buds, although the largest buds have a greater individual potentiality, producing the highest averages of leaf number and area, and also being associated with spurs which have the least leaf drop during the season.

No relation was found between bud size and internodal space.

RELATIONS BETWEEN SIZE CHARACTERS OF LEAVES AND AXILLARY BUDS

At the end of the growing season of 1946 a survey was made of the size relations between leaves and axillary buds on the current year's shoots that had originated from the terminal buds of the same shoots of which the lateral growth has been analyzed. The data are summarized in table 4.

By grouping the total number of 275 buds of 25 terminal shoots of Stark into size categories, the smallest size (group A) was predominant with 49 percent, while the largest size (group D was represented by only 5 percent); 27 percent were of group B and 19 percent of group C. The total area of subtending leaves amounted to 6,280 cm.^{2} The

TABLE 4.—*Relation of leaf area to size of axillary buds on 25 terminal shoots, 1946* [1]

Buds and subtending leaves	Stark					Northern Spy				
	Bud group					Bud group				
	A (1–10 mm.^{2})	B (10–20 mm.^{2})	C (20–30 mm.^{2})	D (30–40 mm.^{2})	Total	A (0–1 mm.^{2})	B (1–5 mm.^{2})	C (5–10 mm.^{2})	D (10–15 mm.^{2})	Total
Buds:										
Total number	[1] 134	74	54	13	275	34	[1] 80	55	8	177
Percent of total	49	27	19	5		19	45	31	5	
Subtending leaves:										
Total area (cm.^{2})	1,578.47	1,991.75	2,116.63	592.93	6,279.78	773.69	2,312.52	2,079.52	378.74	5,544.47
Percent of total	25	32	44	9		14	42	37	7	
Average leaf area per bud (cm.^{2})	12	27	39	[1] 46		23	29	38	[1] 47	

[1] Maxima.

largest leaf area, 44 percent, was associated with buds of group C, and next, 32 percent with group B, while 25 and 9 percent of leaf area corresponded to the extreme bud sizes of groups A and D respectively. However, the ratios between total leaf area and bud number of the respective groups proceed progressively from 12 cm.^{2} in group A, to 27 cm.^{2} in group B, to 39 cm.^{2} in group C, to 46 cm.^{2} in group D—showing the direct relation of leaf area to axillary bud size.

From the terminal shoots of Northern Spy results similar to those of Stark were obtained. The average of leaf area within the single groups gradually increased from 23 cm.^{2} to 47 cm.,^{2} corresponding to size increase of the axillary bud.

It is noteworthy that while the relationship between area of the subtending leaves and bud size proved to be inverse during the early stages of shoot development, the relationship was direct on the fully matured shoots.

Only relatively few leaves (about 10 percent on both Stark and Northern Spy) had abscised at the time when leaf and bud areas were measured, on August 31. Even the most basal leaves still persisted. In spite of the drought prevailing from the middle of June until October, the shedding of leaves was restricted to a minimum, whereas in the preceding year about 50 percent of the foliage fell prematurely. This unusual fact may be attributed to physiological and morphological correlations caused by drought which prematurely halted shoot extension in 1946 and reduced the output of leaves to half of that in the 1945 season. Drought may contribute to early leaf fall, if it occurs after a period of rapid growth which is essential for the elongation of shoots. A distinct inverse correlation seems to exist between persistence of foliage and shoot length, according also to observed conditions of a variety of bushes and trees outside the orchard.

The failure of bud development at the basal region of the shoot has been attributed to its usually early leaf loss. It is true that these characteristics mostly coincide. However, despite the long persistence of the basal leaves in 1946, the usually budless area was hardly reduced.

BUD BREAKING IN RELATION TO BUD SIZE AND POSITION ON STARK TREES

Terminal flower buds open a few days earlier than purely vegetative buds of the same tree. Their flower-forming character is indicated mostly by a larger size and can be determined in advance. The sequence in which lateral vegetative buds break is likewise significant in indicating their future productivity. To a certain extent it seems correlated with the quality of lateral shoots that they will produce. The buds which are the first to break along the shoot axis are mostly those which produce the largest spurs. Roberts [8] attributed the difference in time of breaking to the more or less advanced stage of bud development at the end of the preceding season. However, equal-sized buds of a twig do not necessarily break simultaneously, nor are they always of equivalent potentiality.

[8] See footnote 2, p. 239.

The sequence of bud break does not precisely correspond to bud size, although both factors frequently coincide. Generally, one of the four most apical buds opens first, regardless of size. Among them, the second subterminal is the most likely to break first. Evidently, polarity is another decisive factor. Therefore, the time of breaking is an index of the bud's potentiality only when optimum size and position coincide. Otherwise, these two factors seem to compete with each other and finally the buds in apical position will gain predominance. If a subterminal bud is of equal size with a center bud on the same shoot, the former will produce better growth. However, when a small apical bud has to compete with a maximum bud of the center, the latter may surpass it. Therefore, the performance of the individual buds as evidenced by observation is not always directly correlated to the initial size of the bud and cannot be infallibly forecast. Although a vegetative bud may indicate by its size the inherent potency acquired during the last season, its actual performance is determined also by its position on the shoot axis. Only when large size coincides with a privileged position of the bud is the spur production optimal. Even a smaller apical bud can successfully compete with a larger lower bud, at least in spur length if not also in spur leaf number. Besides this effect of a reciprocal relationship between the inner structural bud development and the current distribution of axial gradients, the lateral growth of apical buds is also benefited by a prolonged seasonal growth period.[9]

CONCLUSIONS

The facts indicated in this study give additional evidence to the concept of size relations between leaf and axillary bud. The ultimate size attained by the bud in late summer and fall is correlated with the size of its subtending leaf. Only on young shoots an inverse relationship exists between the initial bud size and the size of the subtending leaf. This fact may be understood as a consequence of the downward movement of a stimulus initiated in the terminal bud at the time of breaking and therefore first available to the bud primordia which are next to the apex. The stimulus may be of hormonal nature which specifically promotes bud development during its earliest stages, while leaf size depends mainly upon nutritive factors that become effective somewhat later. The sequence of bud breaking indicates a similar primary dependence on the availability of a growth stimulus produced in the terminal bud, although more complex conditions are involved in this phase, such as distribution of axial gradients and the quantitative nature of inner bud structure established during the previous growing season. While large buds of a twig tend to produce more large, many-leaved shoots than do small ones, the final production of a bud depends not only upon its quantitative inner development of primary structures attained at the end of fall, but also on the availability of a specific growth stimulus after the rest period.

[9] The data of bud breaking and subsequent growth were recorded for each single bud on the 25 twigs of both Stark and Northern Spy. The tables which illustrate and support the foregoing statements are too numerous to be included in this paper.

SUMMARY

The results of this study to determine the growth potentialities of vegetative buds on apple trees show:

(1) The development of bud and leaf sizes on the current year's apple shoots is not correlated during the early stages of growth. Only after about 6 or 8 weeks of seasonal growth does a direct relationship between the area of subtending leaves and their axillary buds become evident.

(2) Bud size is a fairly dependable measure of the number of leaf primordia that are differentiated in the bud at any given time.

(3) The frequency of dormancy among lateral buds is inversely correlated with increasing bud size.

(4) The total production of number and area of leaves on lateral shoots is directly related to the size of their parent buds, according to averages derived from group measurements.

(5) Shoots with largest total leaf area are least affected by premature leaf drop during the season.

(6) Though the sequence of the lateral bud breaking on a shoot at the beginning of the season indicates the inherent potentiality of each bud, it does not unrestrictedly forecast their ultimate spur production. The position of the bud along the axis is another essential factor that determines the actual performance of a bud, and may interfere with its growth potentiality as indicated by size.

A STUDY OF UNCONGENIALITY BETWEEN PEACHES AS SCIONS AND THE MARIANNA PLUM AS A STOCK [1]

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INTRODUCTION

Although quality of fruit, as exemplified in color, texture, flavor, and other characteristics, is important to the user, it is not essential to plants for their natural reproduction. The quest for fruit of better quality has led to the selection of individual trees within their parent species, and to perpetuate these varietal selections methods of grafting the chosen varieties onto seedling understocks have been developed. When combinations of closely related plants are used, the graft unions are usually satisfactory, but when interspecific grafts are attempted, the results are not always successful.

Because of the resistance of the Marianna plum to the virus causing peach rosette, a study was made to determine the factors underlying the generally unsuccessful union of peach varieties with this clonal plum understock. The results are presented in this paper.

LITERATURE REVIEW

Argeles (*1*)[3] has published an excellent review of the literature on compatability of pome and stone fruits. The literature referred to in this study will, therefore, be confined to that which has a bearing only on the problem under consideration.

Problems of affinity have existed ever since propagators began using a rootstock of one plant and a scion of another. Chang (*4*) states that in 500 A. D. Chia recorded that plum (*Prunus salicina* Lindl.) could be successfully grafted on peach stocks (*Prunus persica* (L.) Batsch) but that peach usually failed to grow on plum stocks.

According to Hedrick (*6, p. 273*), the Marianna plum "is from either a self or cross-fertilized seed of *Prunus cerasifera*, if the latter the other parent must have been some native species, the particular

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[3] Italic numbers in parentheses refer to Literature Cited, p. 266.

variety possibly being Wild Goose, one of the Munsoniana plums." Waugh (*16*) agrees with Hedrick as to the hybrid origin of Marianna, but he believes it to be a Myrobalan-Chicasaw hybrid.

The readiness with which the Marianna plum roots from hardwood cuttings accounts for its early use as an understock. Marianna was introduced by Charles N. Ely at Smith Point, Tex., in 1884, and by 1888–1889 Smith (*15*) reported its use as an understock for peaches in Georgia and Maryland. In connection with his studies on peach yellows and rosette, Smith recorded that of 124 peach trees of Old Mixon, Early Crawford, Late Crawford, Mountain Rose, and Beer's Smock budded on Marianna plum stocks in August 1889, 24 were dead and 53 were dwarfed and yellowish as though suffering from defective nutrition, on September 18, 1890. None, however, showed any signs of yellows. As Smith's attention was concentrated on virus disease problems, he did not follow up the affinity problems which he encountered.

In 1919 studies of the present writer (*9*) confirmed Smith's finding that the Marianna plum is resistant to the rosette virus.

Booth (*2*) appears to be the next research worker to record compatibility difficulties with the Marianna plum as a rootstock for peaches. He stated that the peaches grew much more rapidly than the plums, and that at the end of 2 years the trunks of the trees were twice as large above the point of union as below. During the hot, dry weather of the second season the peach tops wilted for several days, revived during the night, but finally died.

Crafts (*5*) and Mendel (*12*), working with herbaceous and woody materials respectively, came to the conclusion that anatomical differences between stock and scion are the real reasons for lack of affinity. They hold that symptoms of uncongeniality may appear without any associated structural weakness being apparent.

Bradford and Sitton (*3*) and Proebsting (*13*, *14*) believed that lack of compatibility may be caused by structural weakness throughout the regions of the union. In the writer's studies such weakness was not substantiated by breaking of the peach scions from the plum stocks, such as Proebsting (*14*) found to occur when apricots were budded on plum stocks. The xylem tissues were well developed in the case of both the congenial unions of plum on plum and the uncongenial unions of peach on plum. Many similar unions have been cut longitudinally for less detailed study, and good development of the xylem was observed. In no instance has the writer found in peach on Marianna plum a union of parenchyma cells only, such as frequently develops in unions of pear on quince or apricot on plum. In some cases phloem tissues have turned inward and occupied areas normally filled by xylem tissues, but in no case were the xylem unions sufficiently reduced in area or strength to cause breaking at the unions while the plants were growing in the nursery rows. It is therefore apparent that the uncongeniality of these peach-plum unions is not due to or directly associated with failure of the xylem tissues to unite.

MATERIALS AND PROCEDURE

During 1920 and 1921 numerous Marianna plum stocks were budded to peach at the Georgia Experiment Station. When some of these trees showed sudden wilting in the nursery rows they were dug and

examined and the plum roots were found to be dying or dead. In 1922 these studies were transferred to the Tennessee Experiment Station, and the tests were repeated with hundreds of trees. The results were similar on the Tennessee soils. It was suspected that soil organisms might be attacking the Marianna plum roots. However, detailed pathological studies made by the writer failed to disclose any parasitic organisms associated with the dying or dead roots. The results of these studies (*10, 11*) indicated that the failure of unions was caused by some type of uncongeniality between the stock and scion varieties. From 1919 to 1925 the peach scion varieties Arp, Belle of Georgia, Elberta, J. H. Hale, Heath Cling, Hiley, Mayflower, and Triumph were repeatedly tested on Marianna plum clonal stocks. In no case was good affinity established.

In preparation for anatomical studies of the peach-plum unions, propagation was confined to budding because it has been frequently observed that wounding of xylem tissues of *Prunus* results in excessive gum formation. If gum exudes into the space between the stock and scion it may retard union even in compatible combinations. In budding, care was taken to cut the buds so that only phloem, cambium, and undifferentiated xylem cells were lifted from the peach bud sticks. In making the T-shaped opening in the plum stock for the insertion of the bud, care was taken not to cut into the xylem, thus reducing the possibility of gum formation from wounded xylem tissues of the stock. If gum formation subsequently developed, it was assumed that it was the result of physiological abnormalities. Using a similar technique, other unions were made in which peach seedlings served as the understocks and Marianna plum buds as the scions. These were reciprocals of the uncongenial combinations. Buds of the Methley plum were also put in peach seedling stocks, because this had proved to be a vigorous growing variety when budded on Marianna plum stocks.

Each bud, after it had been inserted and wrapped with rubber budding strips, remained dormant until the following spring. Those combinations intended for anatomical studies were then cut back to the inserted buds, thus making them the terminal buds of each plant. In most cases the inserted buds grew well during the early part of the summer, but by midsummer some of the peach tops began to show evidence of uncongeniality. After the symptoms of a number of these uncongenial and congenial combinations had been recorded, the region of each union was prepared for sectioning. Sections ranging from 16 to 40 microns in thickness were cut with a sliding microtome. Those from 20 to 30 microns in thickness were more complete across the entire union. The fresh sections were washed and stained in haematoxylin and safranine. After it had been destained the material was run up through the alcohol changes to xylol and mounted in balsam. Since the fresh sections did not furnish suitable material for detailed microscopic study, similar unions were placed in hydrofluoric acid until they were desilicified. They were infiltrated with celloidin, according to standard methods for woody materials. These unions were soft enough to cut yet did not crumble when sectioned at 20 microns.

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RESULTS

Microscopic examination of sections of congenial unions such as Methley plum on Marianna plum stocks indicated that the unions were well developed throughout. Plate 1 shows a section of a congenial union. There is some evidence of gum formation in the dark-colored areas at the union, but these gum pockets occupy a relatively small proportion of the union. The xylem is continuous from the stock to the scion. The phloem of stock and scion has united normally, and thus ample sieve tissue to transport elaborated foods from the scion to the stock is assured.

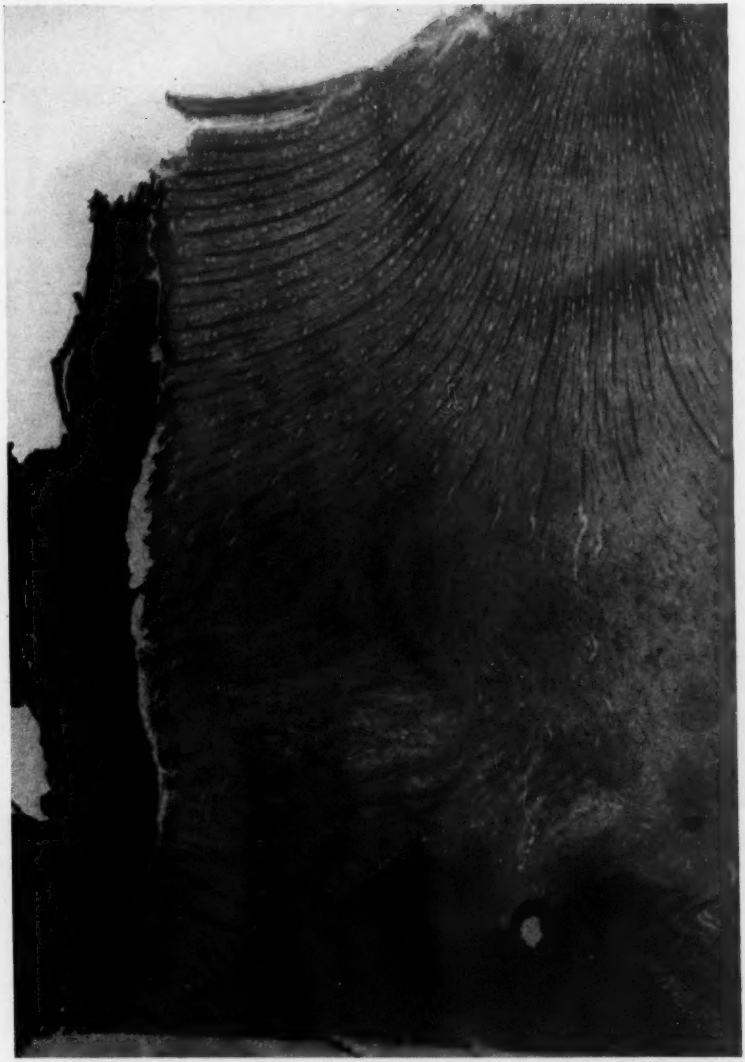
Plate 2 shows the union of all the tissues from the plum stock below to the plum scion above. There is no more distortion of the tissues than might be expected of any inserted bud, as it orients itself from the lateral position where first inserted on the stock, to a more vertical position when it develops into a terminal shoot after the stock is cut back to this bud. In all of the congenial unions, neither gum nor cork was found interfering with normal tissue bridging of the stock to the scion.

Assuming that equal care was used in making all unions, the presence of sizable pockets of gum at the union is evidence that factors other than the wounding of the stock xylem or the desiccation of the cells near the cut edges of the stock and scion (bud) are responsible for gum formation. Sections of the uncongenial unions of peach on Marianna plum were conspicuous for the presence of gum in the region of the union.

Plate 3 shows a section of an uncongenial union of peach, budded on Marianna plum. At the left side of the section the phloem has failed to unite. Both stock and scion phloems have turned inward and developed barriers to the union of the respective xylems. The enlargement in diameter of the scion above the union is evidence that elaborated food was stored there. This was probably caused by failure of the stock and scion phloems to unite and provide sieve tissue continuity across the union. The twisted masses of xylem, seen in plate 3, appear to be mechanically strong, for such combinations do not break at the union. Although apparently strong, the distorted xylem tissues probably do not function normally as compared with xylem in unions of congenial combinations, such as that illustrated in plate 2.

The failure of elaborated food to diffuse down across the graft union could bring about root starvation. A hint that this may be the primary cause of the observed secondary manifestation of wilting is given by the enlargement of the peach scion just above the union, a phenomenon frequently observed in uncongenial peach-plum combinations. The studies of Heinze, Parker, and Borthwick (7) substantiate this theory. They found that in grafts of Red Kidney bean on soybean the Red Kidney bean scions supplied only a portion of the total leaf surface; therefore, the soybean leaves on the stock below the graft union supplied sufficient elaborated food to nourish the roots, and no lethal symptoms of uncongeniality appeared.

No symptoms of uncongeniality developed on any of the combinations when plums were used for scions and peach seedlings for stocks;



Cross section of a congenial union of Methley plum scion (above) on Marianna plum stock. Note continuity of xylem and phloem from stock to scion, and the uniformity in diameter above and below the union.

Cross section of a congenial union of Methley plum scion (above) on Marianna plum stock. The continuity of xylem and phloem from the stock to the scion is complete on both sides of the section. Note also the continuity of ray tissues from the pith region across the entire xylem and phloem, assuring free lateral transport.

xylem and phloem from the stock to the scion is complete on both sides of the section. Note also the continuity of ray tissues from the pith region across the entire xylem and phloem, assuring free lateral transport.

Cross section of an uncongenial union of peach scion (above) on Marianna plum stock. The xylem shows sufficient continuity from stock to scion to provide for movement of water and mineral nutrients and to assure reasonable strength. Failure of stock and scion phloems to unite and overgrowth of the scion tissues just above the union indicate lack of continuity of the phloems and inability of elaborated food materials to be translocated from the scion to the stock.

Cross section of an uncongenial union of peach scion (above) on Marianna plum stock. Although the union of stock and scion xylems is fairly continuous, the stock and scion phloems show little evidence of uniting and appear to be invading regions normally occupied by xylem. Enlargement of the scion tissues above indicates that elaborated food materials are not diffusing from the scion to the stock.

Cross section of an uncongenial union of peach scion (above) on Marianna plum stock. Xylem union, although far from normal, is sufficiently complete for strength and for water and mineral nutrient transport. The inward curling of the stock phloem, at the lower left, and the wide area of exposed xylem indicate failure of scion and stock phloems to unite.

Cross section of a congenial union of plum scion (above) on Marianna plum stock. Continuity of both xylem and phloem from the stock to the scion assures strength of the union and free movement of water and mineral nutrients upward and elaborated food materials downward. Such unions never developed abnormalities of either the scion or stock.

Cross section of an uncongenial union of peach scion (above) on Marianna plum stock. At the right center, the two inrolling masses of stock and scion phloem appear to have avoided contact. The sizable space between the stock and scion phloems leaves exposed xylem, which appears to be providing its own protection through the formation of gum. (Note the discolored xylem in this region.) The extensive development of phloem at the top of the illustration indicates the piling up of elaborated food materials above the graft union.

in fact, of all the combinations studied, peach on Marianna plum understock seemed to be the only one that was truly uncongenial.

In this study there was no difficulty in prolonging the life and growth of peach scions when budded into Marianna plum scaffolds if some plum branches with leaves were left on the stock to nourish the roots. Such top-worked peach branches have been grown on Marianna stocks until they bore normal peach fruits. However, such dual-headed trees are not practical for the use of orchardists.

In combinations in which the scion was entirely peach and the stock was Marianna plum, sections throughout the extent of the union disclosed little or no phloem union. This is illustrated in plates 3, 4, and 5. Phloem cells had been produced by the respective cambia but instead of intermingling, the respective phloems remained separate. In plate 4, which shows an uncongenial union of Marianna plum stock and peach scion, it will be seen that the phloem of the stock has not united with that of the scion on either side of the section. The increased thickness of the scion phloem above is indicative of the accumulation of elaborated food materials. In the uncongenial union illustrated in plate 5, the plum stock phloem at the lower left has turned inward and appears to terminate against masses of developing stock and scion xylem. The peach scion phloem is not complete in this section, but its direction at the upper left indicates that it did not unite with the stock phloem.

Why there is a union between the peach and plum tissues in the xylem and not between the respective phloems is still an unsolved problem. Kostoff (*8*) attributes the failure of union of certain herbaceous plants to the formation of chemical substances which inhibit the passage of food materials across the union. If such substances as precipitins, antigens, etc., are the causal agents in the peach-plum uncongeniality they must be associated with the elaborated food materials moving downward in the phloem, because it is the lack of phloem continuity that is responsible for the failure of such food materials to reach the roots.

From 1940 to 1944 the writer attempted to obtain data on the length of time after budding that each stage in this uncongenial peach-plum union occurs in the field. Seasonal conditions make it difficult to duplicate results, but in the area of Indiana in which the experiments were made the procedure is as follows: The rooted plum stocks are lined out in the field as early in the spring as possible, generally in April; by mid-August the stocks are budded to the various peach varieties, the buds being inserted in a lateral position on the stock, a few inches above the ground. Wound callus develops rapidly, and within 2 weeks the successful unions appear to have united. The inserted peach buds remain dormant throughout the remainder of the growing season and the plum roots are supplied with elaborated food from the plum leaves.

With the advent of cold weather, usually in October, the entire plant becomes defoliated and dormant. The following spring, usually in March or early April, while the plant is still dormant, the entire plum top is cut off just above the peach bud. This changes the inserted peach bud from a lateral to a terminal position. Because of its terminal position the peach bud usually starts growth as soon as

temperature conditions become favorable. Removal of the entire plum stock top down to the inserted peach bud also results in the vigorous growth of numerous plum buds on the stock below the peach bud. Probably because of their more complete union with the plum stock the plum buds grow rapidly into shoots, or suckers. If allowed to take their course the suckers would generally outgrow the peach shoot. This is prevented by removing them, usually when they are only a few inches long.

The removal of these plum suckers throws the entire responsibility for elaborated food production onto the young peach shoot. This results in the very unbalanced condition of a plum stock with more than a year-old root system and a peach top of only a few weeks' growth. In a congenial union (pl. 6) such an unbalanced condition adjusts itself through the rapid growth of the scion into a top capable of furnishing elaborated food for the understock. In the case of the uncongenial combination, however, the sequence of development varies from the normal in several respects. The peach scion growth obtains water and mineral nutrients sufficient for its needs from the plum stock and continues its elongation into a fair-sized whip. This peach top generally appears normal until at least midseason because the elaborated food is ample for its growth. During this time, however, little or no elaborated food has been transferred down across the peach-plum union to the roots, because of failure of phloem union (pl. 7) between the plum stock and the peach scion. This period of 3 to 5 months without food results in exhaustion of available reserves in the plum stock. During this period of declining food supplies in the stock roots, new root growth must progressively lessen. About midsummer a critical stage is reached when no new root growth occurs; as a result, no water and mineral nutrients are obtained from the soil to supply the needs of the expanding peach scion and wilting of the leaves occurs. Previous studies (*10*) indicate that at the time when wilting of the top discloses the extreme manifestation of uncongeniality, death of the roots has generally progressed well up toward the union.

Even though these studies have been conducted with clonal material for both scion and stock, the individual combinations vary somewhat in manifesting the extreme symptoms of uncongeniality. Aside from the time factor, the succession of events is sufficiently uniform to indicate that root starvation finally caused cessation of growth.

SUMMARY

Because of its ease of propagation from cuttings and its resistance to the virus which causes peach rosette, the Marianna plum should be a desirable understock for peaches.

Peach buds of a number of varieties unite readily with this plum stock, and preliminary growth appears satisfactory, but during the second growing season, trees of this combination generally manifest symptoms of uncongeniality. An early symptom, frequently overlooked, is the enlargement of the basal portion of the peach scion just above the union with the plum stock. Somewhat later in the season, the peach leaves begin to wilt, and this symptom is generally followed by death of the entire tree.

Examination of the plum roots at the time the first wilting of the peach leaves occurs, shows that most of the smaller roots have died and the larger ones are dying. Pathological examination of dying and dead roots discloses the presence of no parasitic organisms.

Marianna plum roots of unbudded stocks, as well as those budded to other plum varieties, remained alive when grown in the same soils.

Reciprocal unions in which Marianna plum buds were used as scions on peach seedling understocks developed no symptoms of uncongeniality in the nursery on subsequently in the orchard.

None of the uncongenial unions of peach on Marianna stocks developed mechanical weaknesses such as frequently occur in apricot budded on plum stocks, or certain varieties of pears on quince stocks. Peach-plum unions cut lengthwise disclosed good unions of the xylem tissues.

Cross sections of celloidin-infiltrated tissues in the region of the union disclosed some distortion of xylem and invasion of these tissues by gum. The major defect disclosed by microscopic examination of the sections was the failure of the plum stock and the peach scion phloems to unite. In sections of congenial unions of plum on plum both xylem and phloem union developed.

These anatomical studies indicate that the cause of uncongeniality between peach scions and Marianna plum stocks is the result of failure of the phloems to unite. This lack of phloem continuity prevents sieve tissue continuity; and this in turn prevents elaborated food materials from diffusing down to the stock roots and causes these materials to remain near the base of the peach scion as an overgrowth. This storage of elaborated materials in the scion above the union results in starvation of the stock tissues and generally brings about cessation of new root formation during the second growing season.

Death of the roots from starvation prevents the intake of water and mineral nutrients from the soil. This usually results in wilting of the peach scion leaves by midsummer and finally in death of the entire plant.

LITERATURE CITED

(1) Argeles, G. K.
1937. A review of the literature on stock-scion incompatibility in fruit trees, with particular reference to pome and stone fruits. Imp. Bur. Fruit Prod. Tech. Commun. 9, 115 pp.

(2) Booth, N. O.
1914. Some investigations in grafting. Amer. Soc. Hort. Sci. Proc. (1913) 10: 144–149.

(3) Bradford, F. C., and Sitton, B. G.
1929. Defective graft unions in the apple and the pear. Mich. Agr. Expt. Sta. Tech. Bul. 99, 106 pp., illus.

(4) Chang, W. T.
1938. Studies in incompatibility between stock and scion, with special reference to certain deciduous fruit trees. Jour. Pomol. and Hort. Sci. 15: 267–325, illus.

(5) Crafts, A. S.
1934. Phloem anatomy in two species of Nicotiana, with notes on the interspecific graft union. Bot. Gaz. 95: 992–608, illus.

(6) Hedrick, U. P.
1911. The plums of New York. N. Y. (Geneva) Agr. Expt. Sta. Ann. Rpt. (1910) 18, v. 3, pt. 2, 616 pp., illus.

(7) HEINZE, P. H., PARKER, M. W., and BORTHWICK, H. A.
1942. FLORAL INITIATION IN BILOXI SOYBEAN AS INFLUENCED BY GRAFTING. Bot. Gaz. 103:518–530, illus.
(8) KOSTOFF, D.
1929. ACQUIRED IMMUNITY IN PLANTS. Genetics 14:[37]–77, illus.
(9) MCCLINTOCK, J. A.
1923. PEACH ROSETTE, AN INFECTIOUS MOSAIC. Jour. Agr. Res. 24: 307–315, illus.
(10) ———
[1925.] UNCONGENIALITY, A LIMITING FACTOR IN THE USE OF DISEASE RESISTANT STOCKS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 319–320.
(11) ———
[1926.] FURTHER EVIDENCE OF UNCONGENIALITY IN DISEASE-RESISTANT STOCKS. Amer. Soc. Hort. Sci. Proc. (1925). 22: 231–232.
(12) MENDEL, K.
1936. THE ANATOMY AND HISTOLOGY OF THE BUD-UNION IN CITRUS. Jewish Agency for Palestine, Agr. Expt. Sta., Rehovoth Bul. 19, 46 pp., illus.
(13) PROEBSTING, E. L.
1926. STRUCTURAL WEAKNESSES IN INTERSPECIFIC GRAFTS OF PYRUS. Bot. Gaz. 82: 336–338, illus.
(14) ———
1928. FURTHER OBSERVATIONS ON STRUCTURAL DEFECTS OF THE GRAFT UNION. Bot. Gaz. 86: 82–92, illus.
(15) SMITH, E. F.
1891. ADDITIONAL EVIDENCE ON THE COMMUNICABILITY OF PEACH YELLOWS AND PEACH ROSETTE. U. S. Dept. Agr. Div. Veg. Path. Bul. 1, 65 pp., illus.
(16) WAUGH, F. A.
1898. HYBRID PLUMS. Vt. Agr. Expt. Sta. Bul. 67, 30 pp., illus.

INFLUENCE OF OIL-WAX EMULSION SPRAYS ON SIZE OF MONTMORENCY CHERRIES[1]

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INTRODUCTION

During 1944 in field trials in which oil-wax emulsion sprays were being tested for their efficiency as stickers for fungicides, it was observed that the fruit of Montmorency cherry trees treated with an emulsion designated as No. 82 was substantially larger than that of the control trees.[3] Confirmation of this finding was not possible during the 1945 season at the station orchard; however, preliminary reports from two other experiment stations substantiated this observation.[4] During the 1946 growing season field trials were conducted in an attempt to determine the validity of the above results as well as the basic cause of this effect.

METHODS AND MATERIALS

EMULSIONS

The composition of the emulsions used is given in table 1. Those designated as Nos. 82 and 222 were prepared on a commercial scale by a chemical company at Midland, Mich.; Nos. 410, 411, and 412 were made in the laboratory.

The ingredients of emulsion No. 222 differ from those of emulsion No. 82 in that commercial bodied linseed oil was substituted for the specially prepared oil mix, and Bentonite for the Intracol-S. Emulsion No. 222 has been found to be more satisfactory than No. 82 in respect to both keeping quality and convenience of preparation.

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[3] MILLER, E. J., NEAL, A. L., and GARDNER, V. R. EMULSIONS FOR HORTICULTURAL SPRAYS. Mich. Agr. Expt. Sta. Quart. Bul. 27: 338–351. 1945.

[4] Personal communications.

In an effort to determine the effects upon fruit size of the various ingredients of emulsions 82 and 222, three additional emulsions, designated as Nos. 410, 411, and 412, were prepared and tested.

Emulsion No. 410 contains linseed oil substituted for the oil mix in the emulsion 82 formula. By comparing the results obtained with these two emulsions the importance of the special oil mix in the latter emulsion could be determined. Emulsion 411 has the cobalt and managanese driers added to the linseed oil of the 410 formula, and emulsion 412 has the driers added to the oil in the 222 formula. Thus the necessity for incorporating driers in the oil phase could be determined. If there were no difference between the effectiveness of emulsions 82 and 222 it would be indicated that the specially prepared oil mix, as well as the Intracol-S, was not responsible for the increase in the size of the fruit. A further check on the importance of Intracol-S was obtained by spraying a plot of cherry trees with this compound at a concentration of 80 gm. per hundred gallons of spray mix.

TABLE 1.—*Composition of oil-wax emulsions*

Emulsion No.	Ingredients	Percent by weight	
82	Paraffin wax (Parowax)		26.4
	Oil mix:		
	Linseed oil, body W	2.77	
	Soybean oil, body Z2	5.54	
	Dehydrol, V-11506	2.77	
	Uversol cobalt liquid, 6 percent	.06	
	Uversol manganese liquid, 6 percent	.06	
			11.2
	Span No. 40		4.8
	Tween No. 40		3.5
	Intracol-S		1.0
	Water		53.1
222	Paraffin wax (Parowax)		27
	Linseed oil, body W		11
	Span No. 40		5
	Tween No. 40		4
	Bentonite		1
	Water		52
410	Linseed oil, body W, substituted for oil mix in No. 82		
411	Linseed oil, body W, plus cobalt and manganese driers substituted for oil mix in No. 82.		
412	Cobalt and manganese driers added to linseed oil in No. 222		

FIELD TRIALS

Portions of three commercial and the college cherry (*Prunus cerasus* L.) orchards were used for the field trials under discussion. Since the initial results were obtained when the emulsions were incorporated with the fungicide, the question arose as to what might be expected if they were applied as a separate treatment. Therefore, in orchards Nos. 1, 2, and 4 the emulsion sprays were used independently of the fungicides. The spray treatments are given in table 2. The first application was made when the spur leaves had attained 60 to 70 percent of their final size, approximately 30 to 50 percent of the shoot growth had been made, and the fruit pits had attained their full size and had started to harden. At the time the second application was made in these orchards it was estimated that about 50 percent of the swell in flesh had taken place and 25 percent of the fruits were turning red. Emulsion 82 was used in combination with the fungicide in orchard No. 3 and was applied with the second and third cover sprays

on June 3 and 17, respectively. In each orchard the materials were applied with the regular spray equipment available.

All of the orchards were cultivated with the exception of section 1–B, which was in sod. In order to eliminate as many variables as possible and thus permit a better comparison to be made, the two plots 1–A and 1–B were adjacent.

Fruit samples were obtained by picking at random, from ground level, 2 or 3 lots of 100 cherries each from the individual trees of the variously treated groups. The individual lots were weighed immediately after picking and the average weights of 100 cherries from each tree were used as the basis for statistical analyses of variance.

TABLE 2.—*Influence of oil-wax emulsion sprays upon size of cherries*

Orchard number and data	Treatment [1]		Number of trees	Weight	
	Emulsion No.	Number of applications		Average of 100 cherries [2]	Increase over control [3]
				Grams	*Percent*
Orchard 1–A; cultivated; fungicide used, Fermate; harvest date, July 25:					
First spray application, June 13; second spray application, July 11.	82	1	15	403.7± 6.9	3.8*
	82	2	15	422.8± 8.7	8.7**
	222	1	15	380.4± 8.0	−2.2†
	222	2	15	413.7± 8.7	6.4**
Control			14	388.8± 5.9	
Orchard 1–B; sod; fungicide used, Fermate; harvest date, July 25:					
Spray dates same as for orchard 1–A	82	1	13	406.5± 7.2	8.0**
	82	2	13	427.0± 7.9	12.9**
	222	1	12	416.3± 9.0	10.0**
	222	2	12	427.2± 9.3	12.9**
Control			12	378.3± 7.6	
Orchard 2; cultivated; fungicide used, fixed copper; harvest date, July 25:					
Spray application June 13	82	1	20	462.3±16.8	2.7†
	222	1	18	470.5± 7.5	4.7*
	410	1	8	485.9± 6.9	8.1**
	411	1	8	465.7± 9.3	3.6†
	412	1	9	477.1± 9.0	6.1*
Control			18	449.6± 6.8	
Orchard 3; cultivated; fungicide used, $CuSO_4$–CaO (2–6–100); harvest date, July 24:					
First spray application, June 3; second spray application, June 17.	82	2	[4] 4	373.6±22.5	16.8**
			[5] 7	384.7±17.0	4.1†
			[6] 3	351.3±20.4	2.6†
Control			[4] 5	319.9± 7.4	
			[5] 5	368.7±15.5	
			[6] 2	342.6± 2.1	

[1] Emulsions used at a concentration of 1 percent.
[2] Figures following plus-or-minus sign indicate standard deviation of the mean.
[3] Significance of increase in weight: *, at the 0.05 level; **, at the 0.01 level; †, no significant increase.
[4] Heavy crop.
[5] Medium crop.
[6] Light crop.

TRANSPIRATION STUDIES

A greenhouse study was made to determine the ability of oil-wax emulsions to decrease the rate of transpiration of potted cherry trees, since this may be a factor contributing to the increase in the size of the fruit. Trees about 2 years old were potted in soil at approximately 70 percent of its moisture-holding capacity and the tops of the containers suitably covered to prevent loss of moisture from the soil. The water lost by transpiration was determined by weighing at 2- or 3-day intervals, when an equivalent amount of water was added in order to replenish the original moisture content of the soil. After a standard-

ization period of about 12 days, the plants were sprayed with materials being tested and the efficiency of the treatments calculated from the total water loss of each group as described by Comar and Barr.[5]

In the first experiment 20 potted trees were divided into 5 groups of 4 trees each. The various groups were treated with the following spray mixtures: Group 1, with No. 222, 1 percent concentration; group 2, with Tennessee No. 26, lime, lead arsenate, water (3:3:2:100) plus No. 222, 1 percent concentration; group 3, with No. 82, 1 percent concentration; and group 4, with No. 82, 1 percent concentration, plus the above fungicidal mixture. Group 5 was the control. Each group was given three applications of these materials in the course of the experiment. The second and third applications were made on the tenth and twenty-ninth day, respectively, after the first spraying. On the ninth and twenty-third day all the plants were washed individually by the use of a single-nozzle spray gun attached to the city water supply.

The second experiment of this series was designed to test the efficiency of one application of a 1-percent suspension of emulsions 82 and 222 for an extended period, and also to determine the effectiveness of a 10-percent suspension of emulsion 222. Five plants were used in each of the groups and the experiment was conducted in a manner similar to experiment 1. The temperature, which was kept high intentionally, and the percent relative humidity were recorded by means of a continuous-reading hygrothermograph. The daily maximum temperature and minimum percent relative humidity are shown in figure 2. Since the charts for this instrument are not designed to record temperatures above 100° F., it was necessary to estimate the values between 100° and 110°. Temperatures greater than 110° could not be estimated. On the thirty-third day of this experiment a second application of the 1-percent emulsions was made.

Additional information with respect to the ability of the emulsions or their ingredients to reduce water loss from plant materials was obtained by applying them to apples and determining their loss in weight during storage. The apples were dipped in the emulsion, allowed to dry, and were then either stored at room temperature or in a forced-air dryer at 80° to 85° F. Comparison of the percent loss in weight of the control group with that of the treated groups gave an indication of the efficiency of the materials.

RESULTS AND DISCUSSION

APPLICATION OF SPRAYS TO BEARING CHERRY TREES

The conclusion previously reached that applying oil-wax emulsion sprays to Montmorency cherry trees during fruit development produces larger cherries is confirmed by the data presented in table 2. There was a significant over-all increase in the size of the fruit harvested from the treated trees in orchards 1 and 2.

In orchard 1, part A, there was one group of trees which did not respond favorably to the treatment. This group was sprayed with No. 222, one application, and, although there was a slight loss in weight of the fruit, the value was not significantly different from that of the

[5] COMAR, C. L., and BARR, C. G. EVALUATION OF FOLIAGE INJURY AND WATER LOSS IN CONNECTION WITH USE OF WAX AND OIL EMULSIONS. Plant Physiol. 19: 90–104, illus. 1944.

control. Two applications of No. 82 appeared to be the best in this group; however, there was no significant difference between this treatment and two applications of No. 222. The lower value for this latter group resulted in its not being significantly better than one application of No. 82.

The results obtained with trees growing on sod were more consistent than those of the above group inasmuch as each treatment produced fruit which was significantly larger at the 0.01 level than the control. Although the weight of the fruit from the control group in 1–A was somewhat larger than the corresponding group in 1–B, the difference was not significant. No. 82, two applications, and No. 222, one and two applications, produced equivalent increases; however, the lower value for one application of No. 222 made it equivalent to one application of No. 82.

Trees in orchard 2 were not as uniform with respect to crop or size as were those in orchard 1. For statistical analysis of the data gathered from orchard 2, it was necessary to include all the trees in the various groups irrespective of these variables. The results thus obtained would be expected to be less consistent than if the trees were of uniform size and productivity; however the over-all effect would be analogous to that which would be encountered in many commercial orchards. In orchard 2 the over-all increase of approximately 5 percent in the weight of the fruit from the treated trees, irrespective of the emulsion used, was significant at the 0.05 level. Two of the treatments, namely, 82 and 411, were not significantly beneficial; however, the values for the remaining treatments were sufficiently low to show no significant difference among the treatments themselves. The row of trees treated with emulsion 82 was adjacent to a gravel road, and it is possible that the accumulation of dust upon the leaves interfered with the efficiency of the emulsion.

The application of the emulsion in orchard No. 3 differed from that of the others in that it was incorporated with the fungicide and applied according to the regular spray schedule. The nonuniformity of tree and crop size was encountered again in this orchard. Although the number of trees was smaller than desirable, they were divided into three groups for statistical analysis in order to determine the influence of crop size upon the effectiveness of the treatment. Examination of the data in table 2 shows that a larger increase in the size of the fruit resulted from applying oil-wax emulsion sprays to heavily bearing trees. This increase amounted to 16.8 percent for the experiment under discussion. In the case of medium and light-bearing trees, the increase was 4.1 and 2.6 percent, respectively. The values for the control trees having a light crop appear to be out of line with those of the medium and heavy crop; however, the small number of trees in the former group makes comparison uncertain.

In orchard No. 4 a study was made of the effect of the emulsion sprays upon 53 selections of Montmorency cherry trees. The treatments consisted of one and two applications of emulsions 82, 222, 410, 411, and 412 at 1 percent concentration as well as a 2-percent suspension of 82 and 222. The orchard set-up did not lend itself to statistical analysis because of the small number of trees of each selection in the variously treated groups; however, the results of the weighings appeared to be in accord with those obtained in the other orchards

and no difference was apparent among the strains. It was of interest to note that two applications of a 2-percent suspension of emulsions 82 or 222 caused no apparent injury.

The results from this series of experiments indicate that the specially bodied, expensive oil mixtures used in compounding emulsion 82 may be replaced with the regular commercially bodied linseed oil; and, also, that the cobalt and manganese driers did not contribute markedly to the effectiveness of the emulsions. Likewise, the small amount of Intracol-S, a cationic emulsifier, which might remain in the film is not likely to affect adversely the biological functions of the leaves; furthermore, it does not appear to be the causative agent for producing larger fruits. This belief is further substantiated by the fact that two applications of Intracol-S (80 gm. per 100 gallons) produced no visible foliage injury, and the cherries harvested from these trees, although somewhat larger, were not significantly different from those of the control. It is realized, however, that a complete study of the effect of this material as well as of the emulsions themselves upon plant processes is necessary before this question can be answered with certainty.

EFFECT OF SPRAYS ON TRANSPIRATION

In order to determine to what extent a 1-percent concentration of oil-wax emulsion sprays affects the rate of transpiration of cherry trees, greenhouse experiments were designed to compare the efficiency of Nos. 82 and 222 individually and in combination with a fungicide. The results of these experiments are shown in figures 1 and 2. Since the experiments were not performed under identical conditions, the results are not strictly comparable, and, therefore, only a few general conclusions may be drawn.

It is apparent that treating the trees with the dilute oil-wax emulsion sprays produced a marked reduction in the rate of transpiration for a period of several days. Following this initial period the efficiency decreased until after 10 to 14 days it approached zero. Two subsequent similar treatments produced approximately the same initial effect; however, the efficiency after each succeeding application appeared to decrease at a slower rate than for the one previous (fig. 1). The efficiency of the emulsions was reduced somewhat by incorporating the fungicidal mixture of Tennessee No. 26, lime, and lead arsenate in the spray; however, there was a definite initial retardation in the amount of water lost by transpiration. The cause of the low values for No. 82 plus the fungicidal mixture between the second and third treatment is not understood. Apparently the efficiency of the treatments was not lessened by washing the plants. These studies indicate that the rate of transpiration of potted cherry trees may be reduced for a period of about 1 month by the use of three applications of a 1 percent oil-wax emulsion spray, provided the second and third applications are made about 1 and 3 weeks, respectively, after the first one.

When a 10-percent suspension of emulsion 222 was applied to a group of cherry trees included in experiment 2, the rate of transpiration was reduced 52 percent by the sixth day after spraying. The efficiency remained at this level throughout the eleventh day, at which time the plants were discarded because of injury to the foliage resulting from the high temperatures and the thick layer of oil and wax on the leaves. Injury was not apparent until the maximum tempera-

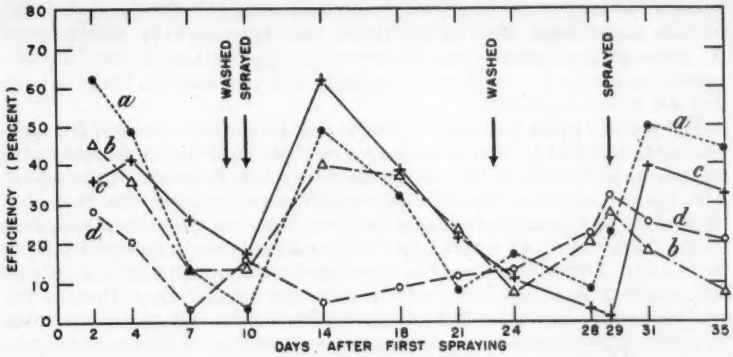

FIGURE 1.—Experiment 1. Curves showing effect of incorporating a fungicide with oil-wax emulsion sprays upon the rate of transpiration of potted cherry trees: *a*, Emulsion No. 222, 1 percent; *b*, emulsion No. 222, 1 percent, plus fungicide; *c*, emulsion No. 82, 1 percent; *d*, emulsion No. 82, 1 percent, plus fungicide.

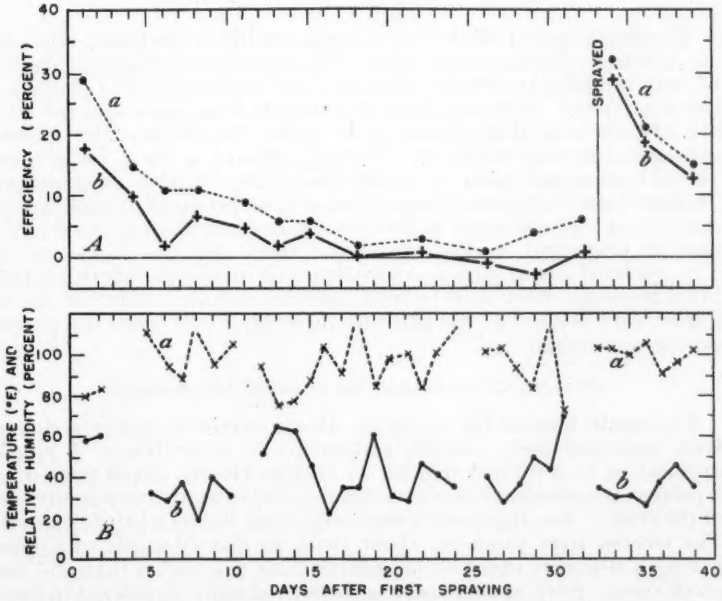

FIGURE 2.—Experiment 2. Curves showing effectiveness of oil-wax emulsion sprays in reducing the rate of transpiration of potted cherry trees: *A*, *a*, emulsion No. 222, 1 percent; *b*, emulsion No. 82, 1 percent. *B*, *a*, daily maximum temperature and *b*, minimum relative humidity.

ture had averaged above 100° F. for at least 6 days. On the eighth day of the experiment the temperature was considerably above 110°. At average greenhouse temperatures the application of this concentrated emulsion to sunflower, soybean and Jerusalem-cherry plants did not result in injury.

The use of apples provided a convenient laboratory method for comparing the efficiency of the emulsions or their various ingredients with respect to their ability to prevent moisture loss from plant materials. The results obtained by this procedure substantiated the fact that emulsions 82 and 222 aided materially in reducing the rate of moisture loss. Application of a relatively high concentrated mixture of Span No. 40 and Tween No. 40 in the same ratio as they appear in emulsion 222 resulted in a loss in weight which was greater than that of the control group; however, at a concentration equivalent to, or less than, that which is contained in a 10-percent suspension of the emulsion, the loss in weight was comparable to that of the control apples. It was also found that a 10-percent aqueous suspension of Intracol-S neither increased nor decreased the moisture loss. It is, therefore, apparent that these emulsifiers did not directly contribute toward the efficiency of the emulsions in reducing the rate of transpiration.

EFFECT OF SPRAYS ON PHOTOSYNTHESIS

The physiological effect of the oil-wax coating on the leaves of plants is a question of prime importance. The application of a concentrated oil-wax emulsion to certain plants has been shown to retard photosynthetic activity.[6] Although such experiments were not conducted with the aforementioned emulsions, it is logical to conclude that photosynthetic reactions might be adversely affected at least for a short period immediately after the application of the dilute emulsion sprays. However, since a 1-percent suspension of these products did not cause a continuous high reduction in the rate of transpiration, it appears likely that no prolonged marked effect upon these reactions would result. The results of a preliminary experiment tend to substantiate this belief, but a thorough study must be made before a definite conclusion can be drawn with respect to the effect of these emulsions upon the photosynthetic processes.

APPLICATION OF SPRAYS TO BEARING APPLE TREES

The results obtained by applying oil-wax emulsion sprays to cherry trees indicated their possible application to other fruit. A single application to a limited number of Grimes Golden apple trees of a 1-percent suspension of emulsion 222 resulted in an increase in the size of the fruit. The application was made about 1 month before harvest. The treated trees produced about twice as many bushels of apples having a diameter equal to, or greater than, 2½ inches than did the check trees. Such an increase was considered quite significant in view of the fact that the total yield from the sprayed trees was approximately 2½ times that of the unsprayed group. This experiment in

[6] BARR, C. G. PHOTOSYNTHESIS IN MAIZE AS INFLUENCED BY A TRANSPIRATION-REDUCING SPRAY. Plant Physiol. 20: 86–97, illus. 1945.

addition to observations made the previous year indicates that there is a possibility of increasing the size of apples by the use of oil-wax emulsion sprays.

SUMMARY

The use of oil-wax emulsion sprays to produce larger cherries has been investigated. A significant increase in the size of the fruit was obtained in three separate orchards by the use of these materials at a 1-percent concentration. Apparently the emulsions may be applied as an individual treatment or in combination with the appropriate fungicides. Limited tests indicate that beneficial results may also be expected from applying these materials to apple trees.

Since no individual component of the emulsions was found to be responsible for the results obtained, the effectiveness of the oil-wax emulsions in reducing the rate of transpiration is apparently the main factor contributing to the production of larger fruit. Although the use of these materials may temporarily impair photosynthesis, it is unlikely that permanent injury would result from their use at low concentrations.

A SIMPLE RAPID METHOD FOR ESTIMATING TOXICITY (ROTENONE EQUIVALENT) OF DERRIS ROOT [1]

By C. PAGÁN, *junior chemist*, and A. J. LOUSTALOT, *plant physiologist and assistant director, Puerto Rico (Mayagüez) Federal Experiment Station, United States Department of Agriculture*

The most reliable criterion for determining the value of any insecticidal plant is the biological assay. By this method the powdered roots are tested on insects reared under uniform and standardized conditions. This method is relatively expensive and time-consuming and, therefore, not adapted for use where large numbers of samples are involved, as in commercial assays or in a plant breeding and selection program.

In tests with the housefly (*Musca domestica* L.) and the Mexican bean beetle (*Epilachna varivestis* Muls.), Jones et al.[2] found no direct relation between the rotenone content of derris roots and their insecticidal value. They reported that the red-color test gave the best chemical estimate of the toxicity of the samples, but it, too, has certain limitations due to the fact that the values obtained are not always in close agreement with the biological assay, as shown in table 1, and the conditions for carrying it out are somewhat exacting.

TABLE 1.—*Chemical analysis of 8 Derris elliptica samples as compared with rotenone equivalent* [1]

Sample No.	Sample [3]	Rotenone equivalent [3]	Transmittance	Rotenone	Total extractives	Red-color value
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	Changi No. 3	18.7	38.8	10.0	22.3	18.2
2	Sarawak Creeping	15.7	45.5	5.7	16.8	13.5
3	do	13.1	46.4	5.4	16.4	12.7
4	Changi No. 3	12.2	48.5	7.3	16.6	13.5
5	do	9.4	51.8	5.3	12.4	10.2
6	Sarawak Creeping	8.5	52.2	5.3	12.6	9.9
7	Changi No. 3	6.3	65.0	3.7	7.8	6.3
8	St. Croix	4.7	71.2	1.8	5.9	4.6

[1] Merriam A. Jones, formerly chemist at this station, participated in some of the chemical analyses reported in this paper.

[3] All samples were grown in Puerto Rico with the exception of Sarawak Creeping (No. 6), which was grown in Guatemala.

[3] The toxicological rotenone equivalent is the percentage of rotenone that the roots would need to contain in order to give the kill actually found. These measurements were made by the turntable method, on houseflies, by W. A. Gersdorff and E. R. McGovran of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.

The main insecticidal constituents found in derris roots are rotenone, deguelin, elliptone, and toxicarol. In preliminary experiments it was found that acetone solutions of these compounds absorb light in the near ultraviolet with a maximum absorption in the neighborhood of

[1] Received for publication October 21, 1947.

[2] JONES, M. A., GERSDORFF, W. A., and MCGOVRAN, E. R. A TOXICOLOGICAL COMPARISON OF DERRIS AND LONCHOCARPUS. Jour. Econ. Ent. 39: 281–283. 1946.

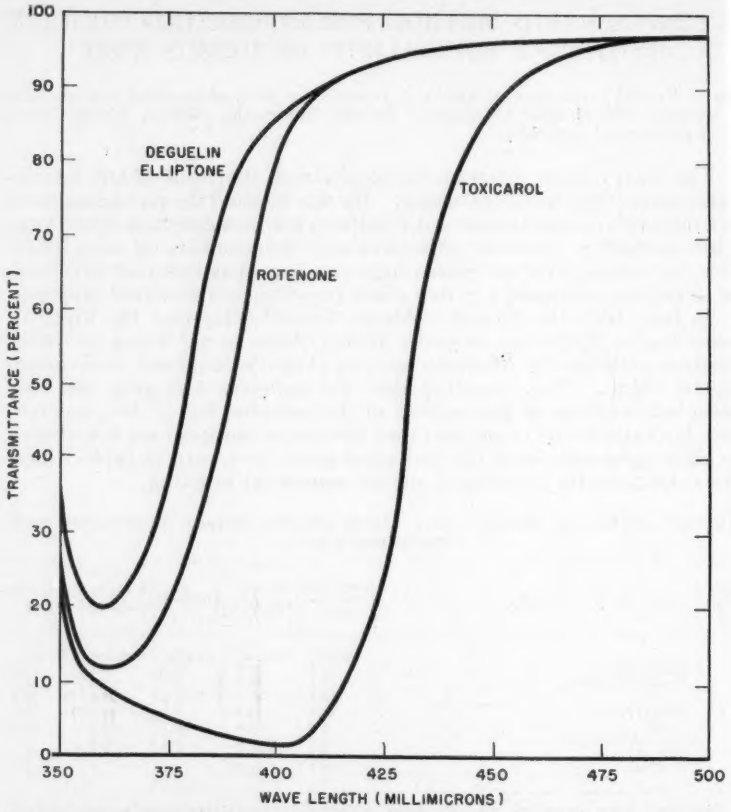

FIGURE 1.—Spectral transmittance curves for deguelin and elliptone, rotenone, and toxicarol.

360 mμ. The spectral transmittance curve of these compounds is shown in figure 1. It will be seen from this graph that the maximum absorption of rotenone, deguelin, and elliptone occurs at a wave length of 360 mμ. Although toxicarol absorbs strongly at 360 mμ, the wave length of maximum absorption is near 400 mμ.

Rotenone is generally considered the most important toxic constituent of derris root and its concentration is the criterion most often used in evaluating the roots. To determine the correlation between rotenone concentration and transmittance values five graded acetone solutions of pure rotenone ranging from 0.2 mg. to 1 mg. of rotenone per milliliter of acetone were prepared. The percentage of transmittance of these solutions was determined at 360 mμ, acetone being used as a blank. The data presented in figure 2 show that when the log of percent transmittance is plotted against concentration of rotenone, there is close agreement with the Beer's-Lambert law. Since it has

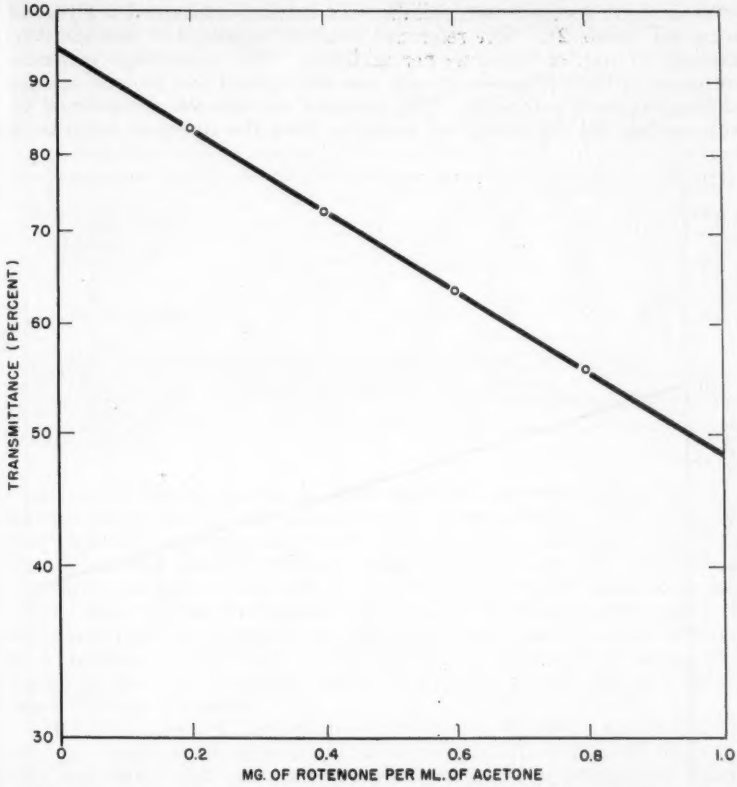

FIGURE 2.—Plot of log transmittance versus concentration of graded solutions of rotenone in acetone.

been shown [3] that there are toxic constituents other than rotenone in derris root, it was necessary to determine whether these constituents, generally designated as rotenoids, absorb light in a spectrophotometer in proportion to their concentration and consequently to their biological rotenone equivalent.

Available for experimentation were 13 samples which had previously been assayed biologically and chemically by Jones et al.[3] Eight of the samples were of *Derris elliptica* (Wall.) Benth. and 5 of *Lonchocarpus* (3 of *L. utilis* A. C. Smith, and 2 of *L. chrysophyllus* Kleinh.). Although it would have been desirable to obtain data on additional samples it was necessary to confine the experiment to these 13 since no other biologically assayed samples were available.

In this experiment the rotenone content was kept constant by weighing various amounts of the eight derris samples and extracting

[3] See footnote 2, p. 271.

with acetone so that each milliliter of solvent contained 0.1 mg. of rotenone (table 2). The reference solution consisted of acetone containing 0.1 mg. of rotenone per milliliter. The percentage of transmittance of these filtered extracts was determined and plotted against concentration of rotenoids. The rotenoid content was determined by subtracting the percentage of rotenone from the rotenone equivalent

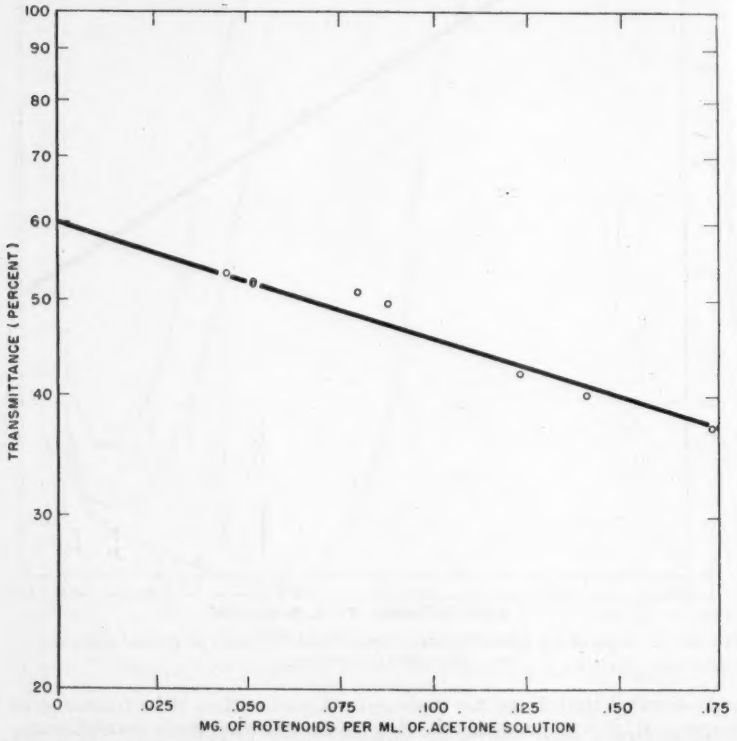

FIGURE 3.—Rotenoid content of eight derris samples plotted against transmittance values.

(see footnotes 2 and 3 in table 2). The data presented in figure 3 and table 2 show a close correlation between the two values. Two of the samples (Changi No. 3) had the same rotenoid content and gave about equal values for transmittance.

These data indicate that when the rotenone content is adjusted to 0.1 mg. per milliliter of acetone and the transmittance value is measured in a spectrophotometer, with an acetone solution containing 0.1 mg. rotenone as a blank, the values obtained give a good estimate of the concentration of other toxic constituents of the roots.

TABLE 2.—*Rotenone equivalent of rotenoids and transmittance values of 8 derris samples adjusted to equal rotenone content (0.1 mg. per milliliter of acetone)*

Sample No.	Sample	Rotenone in roots	Root powder concentration [1]	Rotenone equivalent		Transmittance [4]
				Total [2]	Rotenoids [3]	
		Percent	*Mg. per ml.*	*Mg. per ml.*	*Mg. per ml.*	*Percent*
2	Sarawak Creeping	5.7	1.75	0.275	0.175	37.2
8	St. Croix	1.8	5.56	.236	.136	40.3
3	Sarawak Creeping	5.4	1.85	.222	.122	42.3
1	Changi No. 3	10.0	1.00	.187	.087	50.2
5	do	5.3	1.89	.178	.078	51.4
4	do	7.3	1.37	.153	.053	52.5
7	do	3.7	2.70	.153	.053	52.7
6	Sarawak Creeping	5.3	1.89	.146	.046	53.5

[1] Milligrams of root weighed so that each milliliter of acetone contained 0.1 mg. of rotenone.
[2] Rotenone equivalent per milliliter as determined biologically on houseflies. These data indicate the toxicity due to the 0.1 mg. of rotenone plus the toxicity due to other substances.
[3] These data indicate the toxicity (expressed as rotenone equivalent per milliliter of acetone) after the 0.1 mg. of rotenone is subtracted.
[4] Each sample adjusted to 0.1 mg. of rotenone per milliliter of solvent using 0.1 mg. of rotenone per milliliter of acetone as a blank.

Since the concentration of both rotenone and rotenoids in acetone agreed fairly closely with percentage of transmittance value, the next step was to determine the correlation between biological rotenone equivalent and the transmittance value of the total acetone extractives. Acetone was used as the solvent in these experiments because it dissolved less coloring matter and the toxic constituents were found to be more stable in it than in the other solvents tried, such as benzene and chloroform. A simple and effective procedure for extracting was found by immersing a given weight of suitably ground root in a measured volume of acetone.

The percentage of transmittance of various dilutions of the filtered acetone extracts was measured in a spectrophotometer at 360 mμ until one was found that gave a convenient range in percentage of transmittance between the highest and the lowest samples. The transmittance values of the eight derris samples were plotted against the toxicological value (determined on houseflies) [4] expressed as rotenone equivalent. These data, presented in figure 4, indicate that the transmittance value of acetone extract of *Derris elliptica* is fairly closely related to the biological estimation of toxicity. This apparently applies to samples of *Derris* only, because when the five *Lonchocarpus* samples were tested in a like manner the correlation between percentage of transmittance and rotenone equivalent was not in close agreement. Likewise, the red-color values of these *Lonchocarpus* samples were somewhat divergent from their actual toxicity as determined biologically (see table 3). This is probably an indication that some of the toxic constituents in *Lonchocarpus* roots are different from those in *Derris* roots.

[4] See footnote 2, p. 271.

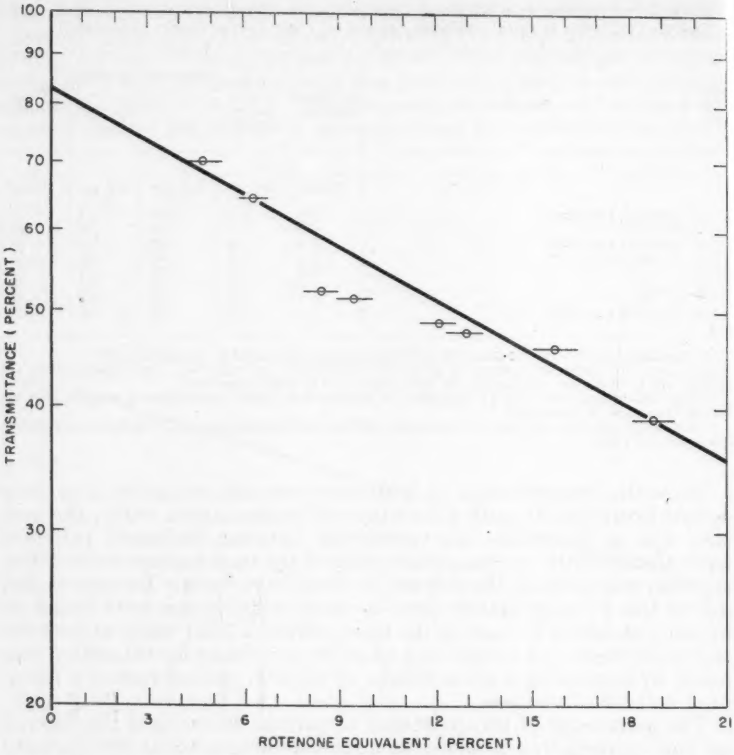

FIGURE 4.—Toxicological rotenone equivalent of derris samples plotted against transmittance values.

TABLE 3.—*Chemical analysis of Lonchocarpus samples as compared with rotenone equivalent*

Sample No.	Sample [1]	Rotenone equivalent [2]	Transmittance	Rotenone	Red-color value	Total $CHCl_3$ extractives
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
9	*L. nicou* (Aubl.) D. C.	9.00	43.5	4.7	8.8	12.4
10	do	8.50	55.0	5.5	6.4	8.3
11	do	7.70	40.1	5.4	10.6	14.0
11	*L. chrysophyllus*	6.70	65.5	5.8	7.0	7.8
13	do	6.80	64.6	5.7	6.2	7.7

[1] The first two samples were grown in South America.
[2] Determined on houseflies by Gersdorff and McGovran, Bureau of Entomology and Plant Quarantine.

On the basis of these data it was felt that the transmittance value of acetone extracts of derris roots might be a fairly accurate measure of the total toxicological value. Such a method would be simple and rapid to carry out and would obviate the more expensive and time-consuming biological assay.

The method finally developed was as follows: Weigh exactly 1 gm. of derris root previously ground to pass an 80-mesh sieve. Transfer to a 125-ml. Erlenmeyer flask and pipette into it 50 ml. of purified acetone. Swirl for a few seconds and set aside for 1 hour or longer with occasional shaking. Cool in the ice box for 30 minutes and filter rapidly taking every precaution to reduce evaporation of the solvent. When the filtrate reaches room temperature, pipette a 10-ml. aliquot into a 100-ml. volumetric flask and make to volume with pure acetone. Measure the percentage of transmittance at 360 mμ in a spectrophotometer using pure acetone as the reference solution. The rotenone equivalent is determined from a standard curve prepared from transmittance values of two biologically assayed derris roots prepared as described above.

SUMMARY

A quick simple method for evaluating the toxicity of derris roots is described. The method is based on the absorption of light by the toxic constituents of the roots, dissolved in acetone and measured in a spectrophotometer at 360 mμ. When acetone extracts of derris samples were adjusted to equal rotenone content and the percentage of transmittance determined, there was close agreement between the known content of other toxic constituents (rotenoids) and the transmittance values. Likewise, transmittance values of graded rotenone solution in acetone determined at 360 mμ followed the Beer's-Lambert law. The transmittance values of total acetone extractives of derris roots were in close agreement with the rotenone equivalents as determined biologically on houseflies. Transmittance values of *Lonchocarpus* root did not correlate with the biological rotenone equivalent.

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